Antigen Presentation Protocol

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ABSTRACT

This protocol details methods for 3-day Antigen Presentation Assay.

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PROTOCOL CITATION

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KEYWORDS

antigen, presentation, Ag

LICENSE

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MATERIALS TEXT

REAGENTS

- LPS/IFNg (including controls)
- RPMI media
- PFA
- PBS
- Wash buffer: DMEM with 0.1M glycine + 10% iFBS
- Stock peptide
- 1x lysis buffer (stock = 5x, with triton, pH = 7.8) in dH2O
- 1M DTT
- CPRG buffer
- Water
- CPRG

CONSUMABLES

- 96-well plates
- 50ml conical tubes

EQUIPMENT

- Incubator: 37°C with 5% CO2
- Centrifuge
- Cell counter
- Plate reader

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

Day 1

1. Prepare a 96 well plate:
   - Label appropriately (experimental condition – LPS/IFNg controls, infection controls, etc.)
   - Duplicate labelled wells for the peptide control

2. Scrape RAW cells and resuspend (pipette up and down 8x).

3. Count RAW cells and adjust concentration to **0.75 million cells/ml**.

4. Add **200 µl RAW cells** to each well.

5. Add LPS/IFNg accordingly.

6. Incubate for **24:00:00** at **37 °C with 5% CO2**.
Day 2

Prior to beginning, check the cell’s media (red/orange = good, yellow = bad).

Prepare 1% (v/v) PFA in PBS at Room temperature (pH 7.4).

Discard supernatant in the 96 well plate and add 50 µl 1% PFA.

Incubate at Room temperature for 00:10:00.

Prepare 2E2 cells while RAW cells are in 1% PFA:

Pour flask into 50 ml conical and centrifuge at 1500 rpm, 00:03:00.

To keep more 2E2 cells growing, add 50 mL RPMI media back into their flask and incubate at 37 °C, 5% CO2.

Count 2E2 cells and adjust concentration to 0.4 million cells/ml.

Add 200 µl wash buffer to each well in the 96 well plate and discard immediately.

Wash buffer = DMEM with 0.1M glycine, + 10% iFBS.

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11.2 Repeat 2x more: Add **200 µl wash buffer** to each well in the 96 well plate and discard immediately. *(Wash 1/2)*

12 Add **250 µl 2E2 cells** per well in ½ of the samples (the non-peptide ones). Add an extra row of 2E2 cells alone for a negative control.

13 Dilute stock peptide (1 µg/ml) **5000 fold** in the 2E2 cells (final concentration: **0.2 nanogram per milliliter (ng/mL)**).

14 Add **250 µl peptide + 2E2 cell solution** to the remaining wells.

15 Incubate at **37 °C, 5% CO2** for **16:00:00 (NO LONGER)**.

Day 3 5m

16 Prepare 1x lysis buffer (stock = 5x, with triton, **pH 7.8** in dH₂O.

16.1 Add **30 µl 1M stock of DTT** in **10 mL lysis buffer**.

17 Prepare CPRG buffer (**pH 7.8**).

18 Centrifuge 96 well plate at **2200 rpm, 00:01:00**.

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Add **50 µl lysis buffer** to all wells.

19.1

Incubate at **Room temperature** for **00:05:00** (up to 20 minutes MAX).

20

Prepare CPRG (recipe below = for each well)
- **150 µl** CPRG buffer
- **20.2 µl** water
- **0.046 mg** CPRG

21

When lysis is done, add **170 µl CPRG solution** / well.

21.1

Incubate either at **Room temperature** or 37°C (37 speeds up reaction to about ~ 20 minutes for peptide samples).

22

Transfer **150 µl colored solution** to a new plate.

Take care not to transfer debris or make bubbles.

23

Take reading at 595 nm or 570 nm.

* To stop reaction to leave overnight, incubate at **4 °C**.